cont A7

60. The method of claim 58, wherein said biological sample is treated to make nucleic acid molecules available for hybridization without purifying said nucleic acid molecules from said sample.--

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REMARKS

Claims 1-20 and 47-57 are pending. All of the claims were rejected on various grounds. These rejections are addressed in order below.

35 U.S.C. §112, ¶ 2

Claims 1-20 and 47-57 were rejected for indefiniteness, on the ground that the claims fail to recite a final process step "which agrees back with the preamble." Independent claims 1 and 47 have been amended so that there is such agreement, and this rejection can now be withdrawn.

Claims 1-20 and 48-54 were rejected for indefiniteness, on the ground that the claims are unclear with respect to the composition of a detection ensemble. Claims 1 and 47, upon which the remaining claims depend, have been amended to require that the detection ensemble be composed of nucleic acids, and this rejection can now be withdrawn.

Claims 1-20 and 48-54 were rejected for indefiniteness, on the ground that the claims are unclear with respect to the reference to the target nucleic acid molecules. Claims 1 and 47, upon which the remaining claims depend, have been amended and this rejection may now be withdrawn.

Claims 1-20 and 48-54 were rejected for indefiniteness, on the ground that it was unclear how providing target nucleic acid molecules in step a)(i) and then detecting target nucleic acid molecules in step b) would result in obtaining any genetic information from the same since "what was provided would then have been detected." Claims 1 and 47, upon which the remaining claims depend, have been amended and this rejection may now be withdrawn.

Claim 4 was rejected for indefiniteness, on the ground that the scope of the limitation from the base claim was unclear. Claim 4 has been amended to specify the type of immobilization claimed and, therefore, this rejection may now be withdrawn.

Claims 5 and 6 were rejected for indefiniteness, on the ground that they did not provide further limitation from the base claim. Claim 5, upon which claim 6 depends, has been amended to depend from claim 8 and this rejection may now be withdrawn.

Claim 7 was rejected for indefiniteness, on the ground that the portion of the method of claim 1 which is intended to be performed *in situ* was not sufficiently identified. Claim 7 has been amended to clearly indicate that *in situ* hybridization occurs after step (b). Therefore, this rejection may now be withdrawn.

Claim 15 was rejected for indefiniteness, on the ground that the claim did not specify the source of the "two different nucleic acid molecules". Claim 15 has been amended to specify that the nucleic acid molecules are from two different regions of the genome of each of the ten viruses. Therefore, this rejection may now be withdrawn.

Claims 16-19 were rejected for indefiniteness, on the ground that the claims did not explain how genetic information is related to information and on the ground that the recitation of "6 or more of ..." was unclear. Claims 16-19 have been amended to address these grounds of rejection.

35 U.S.C. §102: Shuber

Claims 1-6, 8-14, 20, and 47-52 were rejected under 35 U.S.C. §102(e) as being anticipated by Shuber. These rejections should be withdrawn.

The method of claims 1 and 47, the two independent claims from which the other claims depend, is neither taught by Shuber, nor obvious from Shuber, alone or combined with other references. In particular, Shuber does not teach or suggest the use of a detection ensemble with a minimum genomic derivation greater than five.

To adequately explain the fundamental differences between the claimed invention and Shuber, it is useful to first discuss the differences in the conceptual underpinnings of the two approaches, as well as the basic differences in goals of the two methods.

Shuber focuses on scanning a narrow, defined region of a single genome (*e.g.*, several exons of the CFTR gene) in a biological sample for the presence of mutations known to occur in that region. Shuber's primary focus, unlike that of the present invention, is the ability to scan numerous biological samples in parallel. The present invention, in contrast, focuses on testing a single biological sample for (1) the presence of numerous distinct genomes, or (2) for numerous polymorphisms that occur *throughout* an entire genome. Thus, the invention tests a biological sample for the presence of

numerous *unrelated* sequences, while Shuber's invention tests a biological sample for related sequences (usually in the same gene). (By unrelated sequences is meant sequences that do not occur in the same individual organism's genome and sequences that are likely to be unlinked in the genome.) These attributes of the invention enable distinct and important applications not enabled by Shuber's methods.

The ability to test a biological sample for numerous unrelated sequences is significant because it enables powerful applications, such as scanning a lower respiratory clinical sample for dozens of unrelated pathogens that can cause pneumonia, as described in the application. The power of testing for disparate unlinked sequences covering an entire genome also enables forensic applications, high resolution microbial fingerprinting applications, and *whole genome* SNP analysis, as described in the present application.

The key measure of the invention's ability to test a biological sample for numerous unrelated sequences is the minimum genomic derivation of the detection ensemble. The claim term "minimum genomic derivation" is defined on p. 23, with illustrative examples. Claim 1 of the invention requires that the detection ensemble have a minimum genomic derivation of 6 or more. This means that the sequences in the detection ensemble can hybridize to the genomes of *a minimum* 6 distinct organisms (*e.g.*, six different bacterial species). Neither Shuber's examples, nor Shuber's general statements, satisfy this requirement. Furthermore, nothing in Shuber or any of the other references suggests the desirability or feasibility of such high minimum genomic derivations.

The detection sequences in Shuber's examples are *related,* which is reflected in the fact that every group of detection sequences in Shuber has a low minimum genomic derivation. Thus, for example, Shuber's detection sequences generally can hybridize to the genome of one individual or at most the genomes of a small number of individuals – and the minimum genomic derivations are therefore low. This is, in part, a consequence of the nature of the detection sequences used in the examples in Shuber which are generally oligonucleotides comprising one or more single nucleotide polymorphisms (SNPs) or small insertion deletion mutations (relative to the wild-type sequence). As is explained in the present application (page 26 line 4): "...as opposed to ensembles of ID sequences, *an ensemble of SNP probes generally has a minimum genomic derivation of one.* This is because SNP probes can generally hybridize to any genome of the target species with no more than a single base-pair mismatch" (italics in the original).

Consider, for example, the most developed example in Shuber, MASDA 106. Although the detection sequences in the MASDA 106 assay comprise a group of 106 detection sequences corresponding to nine different disease loci, the minimum genomic derivation is less than six, as explained below. Again, the low minimum genomic derivation of MASDA 106, the most complex and most developed example in Shuber, is a reflection of the distinct goals of Shuber's methods as compared to the present invention.

It is important to clarify another point about the detection sequences and samples in Shuber's representative MASDA 106 example. Although, at first glance, one might get

the impression that Shuber tests each patient's sample for each of the 106 detection sequences, this is in fact *not* the case. In fact, each patient's sample is only tested for a small subset of the 106 detection sequences as is explained below.

In the MASDA example, an *a priori* determination is made about the genetic disease that each patient is likely to have. This judgment, generally made on the basis of clinical diagnosis, determines which gene is to be analyzed. So, for example, if a patient is thought to have cystic fibrosis (CF), only the CFTR gene is analyzed, but the beta-globin gene (which would be relevant for thalassemia but not for CF) is not analyzed. The gene (or more usually a small fraction of a single gene) that is determined to be relevant to the patient is then amplified. This purified segment is then tested for the mutations *that are relevant* to the purified segment. That is, if parts of the CFTR gene are purified from a patient thought to have cystic fibrosis, then these gene segments are tested only for the presence of various mutations that occur in the segments (*i.e.*, for the relevant muations).

Testing a purified segment of the CFTR gene for beta-globin gene would not ordinarily make sense. However, this is precisely what is done in MASDA 106, not because it is diagnostically informative, but for reasons that have to do only with the test format. Each purified gene fragment (a representation of the patient's genome -see definition on page 34) is indeed contacted with all 106 ASO sequences. But the only *relevant* detection sequences are those that correspond to the small pre-amplified segment

of the genome of any given patient (*e.g.*, the CFTR gene segments amplified from a patient suspected of having CF).

Thus, in contrast to the present invention, Shuber does not test each sample for numerous unrelated target nucleic acid sequences. In the multiple, parallel screening method of Shuber (exemplified by the MASDA example), each test nucleic acid sample (which is not a raw sample; see new claims 59 and 60) is contacted with multiple probes, almost all of which are known, at the time of testing, to be irrelevant to each individual nucleic acid sample.

Thus, for a patient potentially carrying a CF mutation, Shuber amplifies only CFTR loci and therefore the only detection sequences that fulfill the requirement of claim 1 that detection sequences "can detect target nucleic acid molecules" are the CFTR ASOs (n=66). A single CF patient's sample is thus only tested with CF ASOs that occur in the small fraction of the patient's genome that is amplified. It is these *relevant* detection sequences that determine the minimum genomic derivation.

Thus, to calculate the minimum genomic derivation for an example such as MASDA, it must be clear which detection sequences are relevant to the experiment, which in turn depends on how the biological sample is processed (*e.g.*, for MASDA experiments, which segment(s) are amplified). Then, the minimum genomic derivation of these detection sequences is determined by determining the minimum number of genomes to which they can hybridize. For Shuber's most complex group of relevant detection

sequences, the CFTR sequences (MASDA 106), the minimum genomic derivation is 3, substantially smaller than 6, the lower limit required by claim 1.

To determine the minimum genomic derivation of the CFTR sequences provided by Shuber, one determines the minimum number of genomes that the set of oligonucleotides (17-mers) could hybridize to as a group. Note that, by definition (p. 17), an oligonucleotide "hybridizes to" a genome if, under defined conditions (1M NaCl), the melting temperature of the duplex is less than 12°C below the melting temperature of a duplex of the oligonucleotide and a perfectly complementary oligonucleotide. Using this definition, all of the CFTR oligonucleotides specified in Shuber's example, except for 3, hybridize to a single wild-type genome.

Clearly, all of the wild-type alleles can hybridize to a single wild-type genome. Similarly, all of the mutant alleles with single mutations relative to the wild-type sequence can hybridize to the same wild-type genome. Five of the CF alleles have multiple replacements. Of these, three 1078dPBM, 3659dCM, and 2184AM, have changes (evaluated using the standard 2°/AT base pair + 4°/GC base pair melting temperature rule for oligonucleotides in 1M NaCl) that would prevent efficient hybridization to a wild-type genome under the defined conditions. Therefore, of *all of the CF alleles in Shuber col 18-19 could hybridize to the genomes of 3 individuals* who have the alleles 1078dPBM, 3659dCM, and 2184AM respectively. Thus, for Shuber's cystic fibrosis detection ensemble the minimum genomic derivation would be 3, substantially less than the minimum genomic derivation of 6 required by claim 1.

Thus, the group of detection sequences with the largest minimum genomic derivation in Shuber's examples has a small minimum genomic derivation of three. All of the present claims require a minimum genomic derivation of at least six, which is twice Shuber's highest, and two of the claims (claims 50 and 56) require a minimum genomic derivation greater than 20.

Certainly, nothing in Shuber or any of the other references suggests the desirability or feasibility of such high minimum genomic derivations, or enables their use.

Claim 8, as amended, contains additional limitations that are also not present in Shuber. Amended Claim 8 requires that "*prior* to step (a), nucleic acid molecules *of said sample* are hybridized, simultaneously, with an ensemble of ID probes or SNP probes to yield the probes of step (a)(ii)". The Examiner asserts (page 8, line 1) that Shuber's "probes of step (a) were obtained by first hybridizing a probe pool (ensemble of ID probes) with the sample and then separating them from the sample." However, Shuber does not teach hybridizing an ensemble of ID probes *to the nucleic acids in the biological sample prior to step (a)* as stipulated by amended Claim 8. Rather, Shuber teaches hybridization of a set of ASO probes *to the molecules in a(i), a(iii) and a(iv)*. Amended Claim 8 requires hybridization of an ensemble of ID probes to the unpurified nucleic acid (still in the context of the biological sample (*i.e., before* step a). This is an important part of the invention, as it obviates purification of the target DNA from amplification inhibitors in the sample. Rather than amplifying the target DNA, the ID probes (after separation from the sample) are amplified. Avoiding purification of the sample is

important commercially. This aspect of the invention is also relevant to new claims 59 and 60 discussed further below.

Shuber also fails to teach or render obvious, alone or combined with other references, the inventions of claim 5, as amended herein. Claim 5 has been amended to depend from claim 8, discussed above, in which the first step in the detection process (prior to detection with a detection ensemble) involves hybridizing the nucleic acid molecules of the sample with an ensemble of ID probes (discussed above). In claim 5, as now amended, it is the probes that result from this initial hybridization that are amplified. New claim 58 uses fewer than four pairs of amplification sequences to achieve this, and claims 59 and 60 accomplish this amplification in the context of a raw sample. None of these claimed inventions is taught or suggested by Shuber.

With respect to claim 5, as amended, and new claim 58, Shuber is not amplifying probes resulting from hybridization to the target nucleic acids prior to step (a), as those claims require, but rather amplifies a single gene from purified nucleic acid molecules (for example, the beta-globin gene, with beta-globin-specific primers). This distinction is critical, and goes to the heart of the conceptual differences between Shuber and amended claim 5 and 58 (as it does with respect to amended claim 8, discussed above).

The invention of amended claim 5 and claim 58 amplify the probes resulting from the initial hybridization, amplification being necessary where target molecules are present in the sample in very low numbers, because, inter alia of the impractically slow hybridization kinetics that result from small numbers of probes. The necessity to amplify,

however, presents a different set of practical problems that amended claim 5 and claim 58 solve, and that Shuber fails to address. Amplification of a large number of unrelated genomic target sequences prior to a subsequent detection step would, without the initial hybridization step of amended claim 5 and claim 58, introduce multiplex amplification artifacts that would render the test completely useless. Shuber solves this problem by simply amplifying genomic target sequences from just one gene; if Shuber used the same technique for simultaneous analysis of the combined target sequences of MASDA 106, he would necessarily encounter the aforementioned multiplex artifact problem. Thus, even if col. 9, lines 65-70 through col.10, lines 1-8 of Shuber could be read as suggesting the initial hybridization step of amended claim 8 (without, of course, a minimum genomic derivation of greater than 5), nothing in this passage, or anything else in Shuber, suggests that the resultant probes that will be used in the subsequent hybridization-based detection step should be amplified. It is such amplification, as recited in amended claim 5 and claim 58, that gives the inventions of those claims the power to probe a sample for multiple, unrelated, and rare target genomes, while avoiding multiplex amplification artifacts.

This technology also, unlike Shuber, allows probing of raw samples, in which the target nucleic acid molecules have not first been purified away from the sample. The use of this technology to probe raw samples is now claimed in new claim 59 (dependent on claim 5), and claim 60 (dependent on claim 58). Such probing of raw samples is nowhere suggested in Shuber, and could not be achieved using Shuber's methodology. As is

discussed above, Shuber uses purified DNA, not raw samples, and in each purified DNA sample amplifies only the likely target sequences. It is crucial again to point out that Shuber must limit amplification in this manner, because in Shuber's method multiplexed amplification of many unrelated sequences in each sample would introduce massive amplification artifacts that would completely obscure Shuber's result.

Thus the invention of claims 59 and 60 allows for the screening of a single sample for many unrelated genomes simultaneously. The power of this approach is illustrated by example 3, in which a raw lower respiratory sample is tested for 24 different pathogen genomes simultaneously.

This ability of the invention of claims 59 and 60 to detect target molecules in raw samples has enormous practical and clinical implications. Prior to conducting the test, one need not know, as Shuber does, what the target or targets are most likely to be; any target present that corresponds to an ID probe of the detection ensemble will be detected, because all of the potential targets, not just the one assumed to be present, are amplified.

These claims are clearly novel and unobvious over Shuber.

35 U.S.C. §102: Barany

Claims 1-6, 8-9, 11-15, 19-20, 47-48, and 52 were rejected as anticipated by Barany et al. These rejections should be withdrawn.

Because Barany suffers from the same deficiencies as Shuber, discussed above, all of the arguments previously made with respect to Shuber, above, are repeated by reference as to Barany.

Barany employs a hybridization system which, in terms of targeting sequences in a sample, was admittedly standard at the time; all that was new was Barany's readout method, which is irrelevant to the present claims.

Barany does not disclose or suggest the use of a detection ensemble with a minimum genomic derivation greater than five. In fact, Barany explicitly excludes this type of detection ensemble, which as is discussed above with respect to Shuber, necessarily includes sequences that can hybridize to widely diverse genomes.

Barany discloses none of the limitations of other key dependent claims (claims 5, 8, and 58-60) discussed above with respect to Shuber. Claim 8, as amended, requiring hybridization with an ensemble of ID probes or SNP probes prior to step (a) of claim 1, is not disclosed in Barany, nor is amplification of the probes resulting from the claim 8 hybridization step (claim 5) disclosed in Barany. Raw sample testing (claims 59-60) is also not disclosed.

35 U.S.C. § 103

Claim 7 was rejected for obviousness over Shuber or Barany in view of Bleiweiss. This rejection should be withdrawn.

Claim 7 depends from claim 1, discussed above, which requires the use of a detection ensemble with a minimum genomic derivation greater than 5. Neither Shuber nor Barany satisfies this requirement. Bleiweiss, which distinguishes sub-types of human papillomavirus, does not remedy this deficiency of the primary references.

Claims 16-18 were rejected for obviousness over Shuber and Barany. Claims 16-18 are dependent on claim 1, which as is discussed is not obvious over these references; dependent claims 16-18 necessarily are nonobvious as well.

Claims 53 and 54 were rejected for obviousness over Shuber or Barany in view of Jarvik et al. Claims 53 and 54 are dependent on claim 52, which in turn depends from claim 1. Jarvik et al., like Shuber and Barany, fails to disclose or suggest a detection ensemble with a minimum genomic derivation greater than five. This rejection should be withdrawn.

## Conclusion

In view of the above, it is submitted that all of the claims, as amended, are in condition for allowance, and such action is requested.

Also, applicants request that initialed copies of the forms PTO 1449 that were filed with Information Disclosure Statements filed in this case on November 3, 1999 and November 5, 1999 be enclosed with the next Office Action.


Enclosed is a Petition to extend the period for replying to the Office Action for three months, to and including August 17, 2000, and a check in payment of the required fee. If there are any other charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: August 17, 2000

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